

IAP12 Rec'd PCT/PTO 09 JUN 2006

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SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

<400> 1

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accaactccc agccgtggca ttttattgtt gccagcacgg aagaaggtaa agcgcgtgtt     180
gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgcctcgcac     240
gtcgtggtgt tctgtgcaaa aaccgcgatg gacgatgtct ggctgaagct ggttggtgac     300
caggaagatg ccgatggccg ctttgccacg ccggaagcga aagccgcgaa cgataaaggt     360
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aaaggctaca ccagtctggt ggttggtccg gtaggtcatc acagcgttga agattttaac     600
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<213> *Pseudomonas putida* JLR11

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gcgaggcttg cccgactgtc cggtaaccag cgccatgtcg agcaggcacc gctgttcctg    300
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cagaacgccg cactagcttt cgaggcccaa ggactgggaa tcgtttacat cggcggaatg    480
cgcaaccacc cggaagcgat gtccgaggag cttggcctgc caaacgacac ttctcgctgta    540
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gcgcaatcag tgggtgcttca ccgtgagcgc tatgaggcca ccgaggcaga ggcgggtttca    660
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tcctgggtcca gccaggccgt ggaacgtgta aaaggagcgg attcactgag cggaagacac    780
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<213> *Escherichia coli* K12 nfnB in pET-28(a)(+); pMKS2

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<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc_feature

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<222> (250)..(267)

<223> Cys tags

<220>

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<222> (160)..(177)

<223> His tags

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<222> (268)..(285)

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<222> (996)..(1010)

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat 114
Met Gly Ser Ser His His His His His
1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat 210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp
30 35 40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258
Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala
45 50 55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306
Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu
60 65 70

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caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt	354
Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val	
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gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt	402
Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly	
90 95 100 105	
aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg	450
Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val	
110 115 120	
gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt	498
Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val	
125 130 135	
gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa	546
Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys	
140 145 150	
gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa	594
Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys	
155 160 165	
gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac	642
Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn	
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gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta	690
Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val	
190 195 200	
ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg	738
Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu	
205 210 215	
aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac	786
Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His	
220 225 230	
agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa	834
Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln	
235 240 245	
aac atc acc tta acc gaa gtg taa ttctctcttg ccgggcatct gcccggtat	888
Asn Ile Thr Leu Thr Glu Val	
250 255	
ttcctctcag attctcctga tttgcataac cctgtttcag caagcttcgt catcataggc	948
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<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

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<222> (250)..(267)

<223> Cys tags

<220>

<221> misc_feature

<222> (160)..(177)

<223> His tags

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<221> misc_feature

<222> (268)..(285)

<223> primer

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<222> (996)..(1010)

<223> primer

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Gly Ser Cys Cys Cys Cys Cys Cys Asp Ile Ile Ser Val Ala Leu Lys
35 40 45

Arg His Ser Thr Lys Ala Phe Asp Ala Ser Lys Lys Leu Thr Pro Glu
50 55 60

Gln Ala Glu Gln Ile Lys Thr Leu Leu Gln Tyr Ser Pro Ser Ser Thr
65 70 75 80

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Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg
145 150 155 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser
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Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala
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<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<221> CDS

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<222> (190) .. (225)

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<222> (190) .. (207)

<223> cys tag

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<222> (936) .. (956)

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tgtttaactt taagaaggag atatacc atg ggc agc agc cat cat cat cat cat 114
 Met Gly Ser Ser His His His His His
 1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
 His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
 10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc 210
 Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Ser
 30 35 40

ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca 258
 Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro
 45 50 55

gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat 306
 Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His

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75					80					85							
agc Ser	tgg Trp	gcg Ala	atc Ile	gcg Ala	gcg Ala	gcc Ala	cag Gln	tca Ser	gcc Ala	tcg Ser	act Thr	tcc Ser	tcg Ser	aac Asn	ctg Leu	402	
90					95					100					105		
caa Gln	gct Ala	tgg Trp	agc Ser	gtg Val	ctc Leu	gcc Ala	gtg Val	cgg Arg	gat Asp	cgc Arg	gag Glu	cgt Arg	ctc Leu	gcg Ala	agg Arg	450	
110					115					120							
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140					145					150							
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155					160					165							
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170					175					180					185		
ttc Phe	gag Glu	gcc Ala	caa Gln	gga Gly	ctg Leu	gga Gly	atc Ile	gtt Val	tac Tyr	atc Ile	ggc Gly	gga Gly	atg Met	cgc Arg	aac Asn	690	
190					195					200							
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205					210					215							
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235					240					245							
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250					255					260					265		
agg Arg	atg Met	agc Ser	gac Asp	ttc Phe	caa Gln	cat His	cgt Arg	caa Gln	caa Gln	cgc Arg	gaa Glu	aac Asn	cgt Arg	tcc Ser	tgg Trp	930	
270					275					280							
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285					290					295							
aga	cac	cgc	ttg	cga	gat	gca	tta	aac	acc	cta	ggt	ttc	ggc	ctg	cgc	1026	

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Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg
300 305 310

tga gatagtgaga tatcccatgc ctattcccgc cgccctgaac cggagcacta 1079
atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc 1139
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<212> PRT

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<222> (190)..(225)

<223> primer

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<222> (190)..(207)

<223> cys tag

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<222> (936)..(956)

<223> primer

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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg
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Gly Ser Cys Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys
35 40 45

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Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp
 50 55 60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser
 65 70 75 80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala Ala
 85 90 95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala
 100 105 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn
 115 120 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp
 130 135 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala
 145 150 155 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala
 165 170 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly
 180 185 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu
 195 200 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val
 210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala
 225 230 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu
 245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His
 260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg
 275 280 285

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Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala
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Leu Asn Thr Leu Gly Phe Gly Leu Arg
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<211> 24

<212> DNA

<213> Escherichia coli

<400> 7
ggatccgata tcatttctgt cgcc

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<210> 8

<211> 27

<212> DNA

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<210> 9

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<213> Artificial Sequence

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<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

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